IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : 10/007,459

Applicants : David L. Lewis et al.

Filed : 11/07/2001

Art Unit : 1635

Examiner : Gibbs, Terra C.

Docket No.: Mirus.030.04

For: Inhibition of Gene Expression by Delivery of Small Interfering RNA to Post-Embryonic Animal Cells In Vivo

Commissioner of Patents PO Box 1450 Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.131

Dear Commissioner:

We, Jon A. Wolff, James Hagstrom, Hans Herweijer, David Lewis, Aaron Loomis, and Vladimir Budker, inventor(s) of the above captioned Application, hereby declare as follows:

1. We are inventors of the captioned application.

Jon A. Wolff and Vladimir Budker are the inventors of the process for intravascular injection of nucleic acid into a vessel wherein the volume and rate of the injection results in delivering the nucleic acid from inside the vessel to into an parenchymal cell of claim 1.

Dave Lewis, Jon A. Wolff, Vladimir Budker, Hans Herweijer, James E. Hagstrom, and Aaron Loomis are inventors, separately or together, on claims 11 and 14-18.

Jon A. Wolff and Vladimir Budker are also authors of the cited reference, Zhang et al. Human Gene Therapy 1999, Vol. 10, p. 1735-1737.

- Applicants' in vivo nucleic acid delivery process of claim 1 was conceived prior to the
 effective date of the Office Action prior art references, Zimmer (Methods, 1999) and
 Zhang et al. (Human Gene Therapy 1999).
- 3. We hereby submit photocopies of laboratory notebook pages from the notebooks of researchers working under our direction, dated January 19-22, 1999, and February 10-12 and 19-24, 1999, describing mixing nucleic acid with a polymer to form a complex having a zeta potential that is less negative than the nucleic acid and injecting the complex into a vessel in a mammal in a volume and at a rate sufficient to delivery the nucleic acid to an extravascular cell, prior to the publication date of the Zimmer (Methods, 1999) and Zhang et al. (Human Gene Therapy 1999) cited in the Office Action.

Page 1 of the attached photocopies shows a description of a polycationic polymer used to form a complex with the nucleic acid. Because the polymer is cationic (at the nitrogen atoms), the polymer-nucleic acid complex less negative than the zeta potential of the nucleic acid alone. This polymer, MC00016 (or MC16), was used in the other experiments shown in this declaration.

- Pages 2, 5, 8 of the attached photocopies show descriptions of complex formation between nucleic acid and polymer MC16.
- Pages 2, 3, 5, 6, 8 and 9 of the attached photocopies show descriptions of the injection parameters.
- Pages 4, 7, 10 and 11 of the attached photocopies show effective liver delivery following injection into tail vein.
- It is known to us that the process performed in the notebook pages results in delivery of
 the nucleic acid to extravascular cells as described in the above captioned specification.
- Development of the nucleic acid complex delivery process occurred with due diligence from conception to the filing of the application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

////4//			
		(deceased)	·
Jon A. Wolff	date	Vladimir O Budker	date
Jan & Heyte	8/21/08	- Dul	8/21/08
/James E. Hagstrom	date	Dave Lewis	date
/ /Aaron G. Loomis/ 8/	20/2008	_d	80/15/8
Aaron Loomis	date	Hans Herweijer	date

Mirus Corporation Compound Sheet

MC Number	Lot Number	Date Submitted
MC00016		19/98
Chemical Name		
Mol. Formula	Structure	
Mol. Weight		
Factor		
Compound Class		
Cationic Polymen		
Cationic Polymer Project Particle Formation		
Submitted by		- NOL AS MANNE
S. MonoRom	4~~~~~~~~~	s-s-2
Notebook	1 8	٥
Z-048-Z Amt. Submitted		
Aint. Submitted 1. 2 ~ 5		
Amt. Remaining		
2.5mg		
Appearance red solid		
Approved by		:
Release Date	Lit. Ref	• • •
Elemental Analysis	Analytical	Distribution
Culculated Found	IH NMR 🗸	
	HPLC /	
	Purity	ż
Solubility	Other 12-14,500 diasis	

MIG (SATURS) US. DNA+ PLLDNA IN VIVO

1- 200 Mgs DNA IN 2 Ms H20

2- 200 pgs DNA in 2-10 H20

compact with 134 chances M16 (420 yrs)

3- 200m DMA in 2 mls 400 compact with 3x chare PLL34K-(380ms)

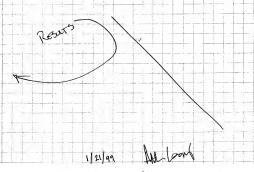
MIX WITH RIWKERS SOLUTION TO 21/2 MIS
HIGH PRESSURE THIS VIEW INTERT

MICH THESSILE THIC DEID TATELY

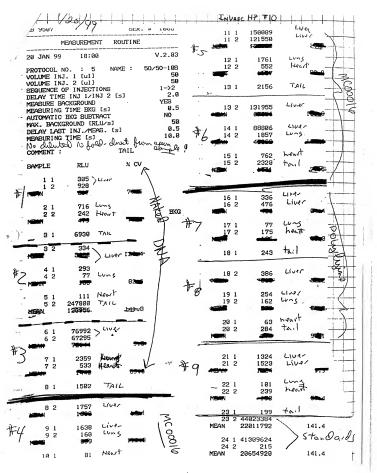
HARVEST @ 24 Hours

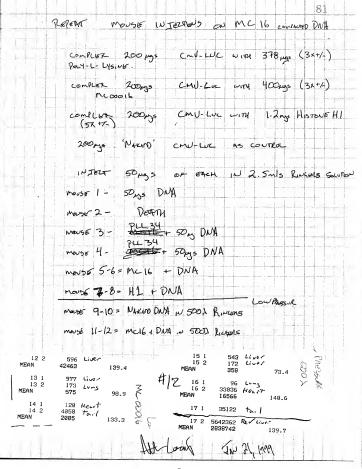
MILE ARE OLD

MILLI TAIL EXPLISSION IS A GOOD INDICATION OF A BAD INTECTION



Project: IM IV InVasc Gut Other:	EXP#: INVACHP \$10	Date: 1/19/	99
Method: LPTail (HPTail) LPPortal HPPortal LP	Gut HPGut IM	Charge to: ATP	PrdDev Other
2.5 Mil's			
Panagrahar(a):	Particle/Compound Being	Tested:	
SEAN	Noted DNA VS		
Delivery Medium: Saline PBS Glu Man H2O	MIRVS MC 16 VS	Dale lun 1 4	
Ringers other	7.44	المالع والمالة	
Stability Issues?	Hazardous Material?	Other Cautions	?
	_	_	
Time Point(s): Z4 Ha HARVEST	-		
# of Samples:	Explanation/Code:		
3 x 3 = 9 total			
AMMALS		•	
End Result: Expression:	******************************	Distribution:	
Luciferase	7		Fluorescence
BetaGAL	•		Radiation
GFP			other
Ligand/SolF	Recep		
Ab Screen			
other			
outer .			
Organs to Assay: LIVER LR / MC	TAIL		*
lotes:	:		
Procedure Notes:		Start Time:	
		Otan Timor	
ndividual Animals:			
Inj Notes			
DrinaL			
p1 - 6-000			
*Z - BAD NAKE	DN4		
#3-04			
*4 ok	inus MC16		
	479		٠.
The GREAT	_	~ Polystyp	
#7 GREAT	4 - 43	7,75	
+ 8 Zme foot them 0.50	K E K J	. /	
79 1.5 then 1.0 mo	we moved - Quickle	1.0	
ocedure Performed by:	-!	Date:	





	EXP#: INVASCHP HI		
Method LPTail HPTail LPPortal HPPortal LPGu	t HPGut IM	Charge to: ATP	PrdDev Other
Researcher(s): S. M. Delivery Medium: Saline PBS Glu Man H2O	A A 3	√ s	
Ringers Other	MC16 VS	16	
1	Hazardous Material?	Other Cautions?	
Time Point(s): 24 Hours	**		
Time Point(s): 24 Hora # of Samples: 6 x Z= /2 Samples	Explanation/Code:	•	
End Result: Expression:		Distribution:	
Luciferase			Fluorescence
BetaGAL			Radiation
GFP			other
Ligand/SolRed	cep		
Ab Screen			
other			
Organs to Assay: Lune	TAIL		
LIVER LA/MC HEART			
Notes:			
Procedure Notes:		Start Time:	1
Individual Animals:			•
ANIMACS 1 - 2's me fast pDNA < 2 - 1's me died during	tquests areas		
PLL <3. 28 Great ing			
MC16 < 5-04			
HI 47 2 ma then 15 min late 8 2 true then in 10 2 10 2 10 2 10 2 10 2 10 2 10 2 10 2	u Émlmore		
trans emoor wil of Ande	, o		
MCILCAS LV SOOM greating	•		
Procedure Performed by:		Date:	

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-	00.74		REMENT	ROUTINE				~ -10 2 , ME≙N	718416 368687	8 Liver 7	1 年日本記	1000
-		1 99 201 NO		NAME :		V.2.03		, _ 11 1	639460	4 Live.	-	(
	VOLUME	INJ. 1 INJ. 2 CE OF IN TIME INJ	[u]] [u]] JECTION	e.	50/	50 50 1->2	6) 1 1 2 MEAN		0 Heart		6
	MEASUR	E BACKGRO ING TIME	DUND BKG [=	7		2.0 YES 0.5		2 1	1467	5 Tail		
	MAX. B DELAY	TIC BKG S ACKGROUND LAST INJ. ING TIME	UBTRACT RLUZ	r =1	,	NO 50 0.5		2 2 MEMO:	48		k32-4	A
1 -	COMMENT			TAIL			#~	3 1 3 2 MEAN	2743 242 1 49 3	Lung	1 18- 5	C
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NI PRE	2 2 MEMN Sue e	1433: 3156 874 3	2 lung &	Z 992.4			#8	5 1 5 2	389 315 349		13:2	100
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	MEAN	62228	3	195=5				7 1	4339	tail	10.6	
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-	5 1 5 2 MEAN		Heart toil	92-4	Lysine		¥9	8 1 8 2 MBAN	446 147 297	Liver	7 5 50	To Pie
*4	6 1 6 2 NEAN	35925 31202 3956 4			þ			9 1 9 2 NEAN	470 404270 20037 0	HIART tail	*41. 1	35 ME
,	7 1 7 2 MEAN	1549 227 888	Lung Herst	185_8		#	D	10 1 10 2 MEAN	219 342 281	Liver	31.0	DWA
	8 1	1289522	Liver					11 1 11 2 MEAN		Luns Heart	3.5	٦
*5	8 2	882382 1 085952	Liver	26.5	W600010		_	12 1	84330	ta:1		
	91 92 NEAN	14264 3845			0							

MC 55 + MC 56 ME NOT SOUBLE IN HEPOS BUPFER - SO ALL FORMULATION WILL BE ORIGINALLY DONE IN DIMSO. DUPLICATE MICE - 100M DWA-PCILUX EACH MOUSE - ROMPACT 200M GACH ACKENT 1+2= 200, DNA + 300gl DM50 -7 ADD 2,5 nls RINGERS M16=1.7x 3+4= 2004 DNA+300, DMSO + MC16-4 (3x curred) Img -ADD 25 mls Riverts 5+6= 200m DNAT 300f DMSO + MC16-5 [3x+] Img - ADD 2.5m/s Rugers 7+8= 200, DNA+ 300, DMSO + MC55 [-3x+] 1mg -> ADD 25 LIS RINGERS 9+10= 200m DNA+300 DMSO+ MC56[-3x+] In -7 ADD 2.5413 RINGERS MCS7=0.5641+12 = 200 n DNM + 300 N DNSO+ MC57 [-3x+] 336 mg -7 ADD 25 m/s Revines 13+14 = 200, DNA + 300, DMSO + MC 58 1-3x+] 324m -> ADD 25-1/3 RWGAR mc 59= 0.79 15+16= 200, DNA+ 300, DMSO+ MC59 [-3++] 474m -> ADD 25 5m/s Ruyers mc60=078 17+18-200pg DNA+300pl DMSO+MC60 F3x+1 468pg -> ADD 25mbs Rungo > FORMULATION PROBLEMS MC 16-5 IS NOT SOUBLE- IT IS A MASS OF LARGE AGGREGATES MC55 FALLS OUT OF SOLUTION WHEN IT HITS RIVIERS MC59 CLOUDS UP WHEN IT HITS RINGERS VESULTS. OVER-NJEGOUS 1- GREAT 11- GREAT 13' GREAT INT 2- LOST SONE FORMLATION 12- GREAT 14- GREAT INT 3- GREAT IT GREAT WI 4- A LITTLE SLOW ~ 10-12-SET STOPPED BREARING 16 - GRAFINI 5- FORMULATION KILLED IT - GOOD INSTERDAND 17 GREAT WI 6- GREAT WITHOUT 18- GRUSATING 7- 2.2 ml our 8- GROW IUT. 10- Grap int - some Proserve HAR FEB 22, 1999

Project: IM IV InVasc Gut Other:	EXP#: InvascHP # 1Z	Date: <u>2/22/0</u>	19
Method: LPTail HPTail LPPortal HPPortal LPC	out HPGut IM	Charge to: ATP	PrdDev Other
Researcher(s): S,M, /COMIS Delivery Medium; Saline PBS Glu Man H2O Ringers Other DMS C		ested: 57 MS9 Vs 158 Mbo	AUD9
Stability Issues?	Hazardous Material?	Other Cautions?	
Time Point(s): 24 Hours - Harvest			
# of Samples: 9 x Z = 18 ANMAG	Explanation/Code:		
		Distribution:	
End Result: Expression:	-	Distribution.	Fluorescence
Luciferase BetaGAL			Radiation
GFP			other
Ligand/SolF	lease.		
Ab Screen	тесер		
other			- 0
Organs to Assay: Live LR/MC	LUNG	KIDNEYS	
SPLEEN	HEART	TAIL	
Notes: HI Ublume TAIL ** WITH DMSD - ANIMAL SHOOK AF	TER INS.		
Procedure Notes:		Start Time:	
5 WK OLD			t
Individual Animals:		A	•
ANIMALS - Inj Hame	t absentin	#17 Great a	MARMA
100 mg p DNA < # 2 Some out 1st had > Major	PZ	FIB CHEEN	mos M.
MIG-4 < + 4 Some of 100 > Some DA	MAG-6 - Spets		
MIG-5 CF5 DEAD > Clots			
MSS <#7 7.2 ml - Blood Clots	ts		
M55 < 187 2.2 ml - Blood Clots spe 88 Grent light Celor Spe M56 < 40 Good ing - Vers Ugls de 400 Good ing - Vers Ugls de	Buch Hots		
M58 < \$11 Great mo convolum > M58 < \$12 Great shakes M58 < \$13 Great > Small cloto	large clut on liver	To	
M58 < \$13 Greek > small cloto		Also Refr. To 79	(GE)
M59 < \$15 > Great vacations > Procedure Performed by: Mark Noble	_	Date	
M. W. Walke	<u>.</u>	7/22/	44

1		2/24/99 Samples have 33rd proper assessed 24"
1	1	INVASE HP \$12

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124/99			mac

1	2/2	4/99				g- , ,		pu	some
<i>i</i> .	9507					SER.	#	1088	3
		MEAS	URE	ENT	ROU	TINE			_
24	FEB	99	Ø9:	09				v.2.	33
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CO	MMENT	:			DR	.M			
SA	MPLE	1	STU						
	1	26050	586	LR >	Liver				
W. W.	2	21630	546			O Ans			
r	3	5847	298	Splen	~	NrF9			
	4	1014	109	Long		DIV)	?		
	5	560	590	Heo_t					
	6	694	63	kudn	us				
	- 7	132	254	tail					
اسس	8	1566	183		liver	- (-		`	
* 2	9	1191	101	mc	Lve	: dama trushe	gd		
	10	5165	558	Spleen		·1-Che	ci 🖫	/	
	11	851	48	Lung		Nake	./ N	v/A	
	12	688	804	Hecut	-	····		<u>.</u> .	474
	13	281	35	Kilne	~				
	14	367	83	tais					

S.M. Complexes

24 How hernest LIVERS TOX delation each ?

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16	8672595 M(
17	3967398 Splen .#3
18	649693 Lms MC 16-4
19	358458 Hemt
20	88135 Kidnes
21	22478 tail
22	20836924 LR
23	14335173 mc +4 =
24	10303277 Spleen M(16-4
25	2205514 Lung
26	319212 Hent
27	435213 kidners
28	34322 tail
29	15893273 LR *6
30	14260842 MX LIVER MC16-5 D duis
31	3568655 Splean ,maly 2 6238338
32	663279 tung
33	212093 Heint
34	2751592 kidners plens
35	7972 tail _e
₩.5	dend-

/		4		111	741	CHVASCHP	*1Z
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1	36	MC-55 ↓ 17862760	LR ·	64	6892050 LR Sciver	∜ (5 92	/
,		15428635	1000	*11 ₆₅	5883566 Mc	93	54733 M C
6 7	38	356868		66	2794699 Sples	94	114178 Splen
Mes		392596	Lung	67	261024 Lung	95	35211 lung
	40	215870	went	68	122821 heart	96	-
	41	75095	tilsen	69	151890 kilnero	97	48360 kilney
	42	2859	tail	70	30516 tail	98	9235 tail
	43	13063372		*1Z71	17868750 LK	T16 1	285662 LL > LIVEY 262457 MC
*8	44	13417665	mr > lim/	72	11401863 ne	2	16426 Splein
Mes	÷45	4958813	Splem	73	4176665 Splein	3	18562 4
	46	785182	lung	74	678634 Lung	5	15567 heart
	47	231348	heart	75	375063 becut	6	9187 kilney
	48	184282	kidnew	76	186018 kidney	7	4811 toil
) —	49	63918	toul	77	5061 tail		mc-60 +17
,		nc-56+		\$13.78	30968612 LR	*178	769642 LR) Liver
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M-56		1342129		81	1440903 Lung	11	15585 hung
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				88	300536 سى		2/24/99
	60	113231	ling				S.M. Challerin
	60 61		heart	89	251567 head		S.M. Cemplerus
		63804		89 90	363527 Kidne	* 1	S.M. Complexes Hish volume 2.5 ml
	61	63804	heurt Kidnes			* 1	Hi sh volume